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TITLE: Role of Nonneuronal Cells in Tauopathies After Brain Injury

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13. SUPPLEMENTARY NOTES

14. ABSTRACT The purpose of this study is to identify how, after mild repeated traumatic brain injury (TBI), specific inflammatory factors (complement proteins) elevated during long asymptomatic prodromal period are responsible for the eventual onset of cognitive deficits and neurodegeneration. We investigate how inflammation leads to accumulation of aberrant tau aggregates, a common downstream pathway directly causing neurodegeneration in many neurodegenerative disease, including TBI. We use a human Tau Tg mouse that models effects of TBI on normal tau expression. This mouse is bred to mice with novel transgenes associated with complement activation: one lacking the "brake" of the complement cascade (C1inh KO) and the other overexpressing C5a. During this 2nd year we produced new data demonstrating a robust effect of C1inhKO affecting the response to TBI in human tau mice, increasing tau and pyknotic neurons as well as a tau kinase which increases tau phosphorylation, supporting a pathogenic role of C1q in tau-dependent injury after TBI. We have demonstrated that TBI causes tau dependent increases in microglia but C1inhKO-injured mice without hu tau, show no changes. In contrast compared to wildtype mice, injury increases GFAP in both C1inhKo and htau mice. We have identified 2 promising glial markers in brain derived plasma exosomes (aquaporin and GFAP)

15. SUBJECT TERMS Glia, microglia, mild traumatic brain Injury, chronic traumatic encephalopathy, complement cascade, neuroinflammation, neurofibrillary tangles, tau, trans-synaptic, phagocytosis

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Table of Contents

1. **INTRODUCTION:** The purpose of this study is to use animal models to elucidate the mechanisms after repeated mild traumatic brain injury (mTBI), leading to neurodegenerative disease, such as chronic traumatic encephalopathy that occur after several asymptomatic months or years. This long asymptomatic period suggests that the brain has strong protective mechanisms against deleterious effects, but that eventually there is failure to compensate. The main pathology thought to cause onset of the disease is accumulation of abnormal aggregates of a protein called tau, which is a pathology common to many neurodegenerative diseases. Chronic aberrant neuroinflammation (dysregulation of astrocytes and microglia), during the asymptomatic period is known to drive tau pathogenesis through activating tau kinases, but the mechanisms remain elusive. We have identified an inflammatory pathway called the complement cascade involving the microglia, which plays an essential role in synaptic pruning, but no model to date has modeled its hyperactivation, known to occur after TBI. Since our preliminary data shows that C1q plays a prominent role in tau accumulation and that these effects are mediated by C5 convertase, we have obtained novel models that will for the first time allow study of these mechanisms. Our data also show that the complement cascade plays a role in tau accumulation that is distinctive and opposite from its role in amyloid accumulation. This proposal investigates the hypothesis that the dysregulation of glia plays a critical role in tau spreading leading to cognitive loss.
2. **KEYWORDS:** Microglia, Astrocytes, tau, complement 5a, serpin, C1 esterase inhibitor, tau kinases, chronic traumatic encephalopathy, post-traumatic brain injury, trans-synaptic, phagocytosis.
3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project during this Period (Year 2)?**
     - **<u>Major Task 2 (Subtask 2 and 3)</u>:** To randomize C5a strains (with our without htau, and htau and C57bl littermates) to TBI or sham.
     - **<u>Major task 3 (Subtasks 1-3)</u>** To perform TBI on the four C5a & littermate groups and age out to 9 months) and perform behavioral testing
     - **<u>Major task 6 (Subtasks 2-4)</u>** To perform TBI on the four Serpin1 & littermate groups and age out to 9 months) and perform behavioral testing

   - **What was accomplished under these goals?**
     - **<u>Major Task 2 (Subtask 2 and 3)</u>:** We have produced and randomized the C5a strains (with our without htau, and htau and C57bl littermates) to TBI or sham.
     - **<u>Major task 3 (Subtasks 1-3)</u>** We have not been able to produce all of these mice within the time frame, resulting in not all of them being aged out. 50% have been subjected to TBI on the four C5a & littermate groups and only 15% have been age out to 9 months and subjected to behavioral testing and we have no data yet on the bigenic. We found that in response to injury, the probe test with Barnes Maze, showed that the C5a made fewer errors but showed fewer entries into target., identifying increased apathy, but not cognition. Therefore this is the first data showing any phenotype of this C5a mice , implicating a role of C5a in injury. (See Figure 1 below). Prevoiius work on this model showed no impact of C5a in a multiple sclerosis model and no phenotype in the absence of any manipulation, which makes it an exceptional model to study the role of C5a in disease. Further we show that in response to injury the htau show significant deficits, unlike the wildtype (See Figure 1 below). The C57 showed reduce entries in response to injury without any effect on errors, suggesting that this variable may be useful to assess injury effects, again potentially reflective of injury associated depression. Also we

show that the C5a have a slightly lower preference for novel object (Figure 2). All mice show longer recovery after anesthesia during TBI (Figure 3), showing that this variable is useful in ensuring the magnitude of each mild TBI, which is important since variations can occur despite controlling for psi and velocity of piston.

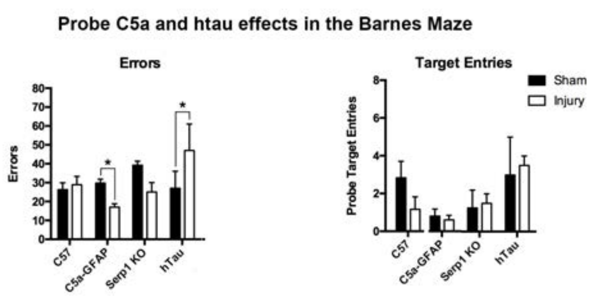

Figure 1:Probe test results of the Barnes Maze. *Htau mice subjected to repeat mild TBI (rmTBI) showed increased errors; but none of the other strains without htau showed this effect. Injury in the C5 and Serpin KO mice caused reduced entries in target hole compare to other strains even without rmTBI, even increasing errors in the C5a reduced. This suggests a potential baseline apathy or depression in these lines.*

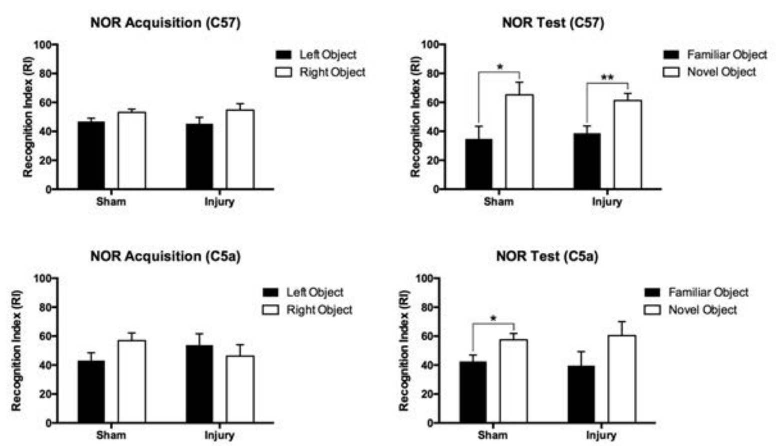

Figure 2: rmTBI effects on Novel Object Recognition in C57bl and C5a mice*. Data show that even after rmTBI C57bl show preference for novel object. However rmTBI causes a slight but significant deficit in the C5a mice.*

- **<u>Major task 6 (Subtasks 2-4)</u>**
- (i) We have performed TBI on the Serpin1 &wildtype littermate groups, but had failed to produce any htau-serpin bigenic mice on the mouise tau background. Each of the litters should have had 25% of each of the four lines to be compared; yet in over 20 crosses, all htau-serpin were lethal. This suggests that there is a major interaction between C1q and mouse tau that is affecting development. (ii) We were able to resolve this problem after one year of not being able to overcome the lethality of the cross, by breeding the Serpin with htau when there was only one copy of mouse tau (half tauKO). Now, although they are randomized, they are not yet old enough to be subjected to TBI. (iii) Although we only have new pilot data for performing behavioral testing in the htau vs serpin KO (Figure 1) we have some preliminary histological data of the cross (Figure 2). Serpin KO mice showed an underlying reduction in entries in the Barnes maze, independent of injury, but no increase in errors (Figure 1). This supports a potential baseline apathy in these mice which is not further worsened by injury.

- (iv) Histological analysis of the cross revealed striking neuropathological effects of rmTBI. Histological data is shown in Figures 3-7. First, Cresyl Violet staining of the brain sections showed a robust interaction between htau and SerpinKO in a pilot group. This cross require removal of one copy of mouse tau to address lethality in the hu tau x C1q containing two mouse tau copies (Figure 2). In these bigenic tau-serpinKO mice, compared to the contralateral side to injury, rmTBI caused a robust loss of CA1 neurons and pyknosis of subicular neurons, supporting a critical role of C1q in human tau-associated neurodegeneration after rmTBI. Although we have yet to have glial analyses of the cross, we do show that huTau Tg mice but not serpin KO show increased microglial iba (Figure 4) and astroglial GFAP (Figure 5). The distribution of the tau kinase c-Jun N terminal kinase (phospho JNK) is altered in the huTau Tg mice, where there is increased staining in both CA3 pyramidal neurons as well as in dendritic areas (Figure 6). Injury in the Serpin-htau mice was associated with bilateral increases in JNK in the SL of the hippocampus (oval), but we do not know if this is injury or serpinKO dependent, because the Serpin KO alone tissue was not sufficiently fixed. Figure 7 showed that in the absence of human tau, there was a robust increase in perivascular tau in serpinKO mice after injury, supporting the importance of C1q – tau interaction in TBI pathogenesis. We are excited to examine the response in SerpinKO/htau bigenic mice.

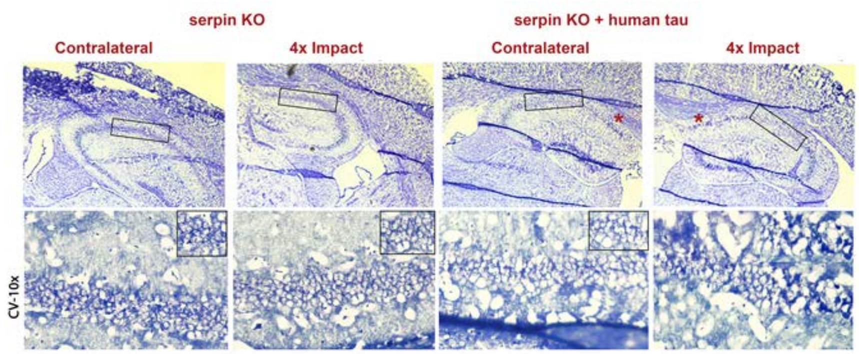

Figure 3. Cresyl Violet staining of brains of serpin KO with and human Tau subjected to mild repeat TBI or sham (pilot n=3*). We observed relatively little effect of injury on the medial CA1 pyramidal neurons of Serpin KO nor did we observe a major change in sham treated human tau compared to sham SerpinKO. However, there was a robust loss of lateral CA1 neurons in rmTBI treated Serpin KO/tau mice. This loss is boxed in the upper panels and magnified in the lower panels, and the boxed area in the lower panels shows further magnification to depict Nissl staining. The CA1medial neurons projecting to the subiculum are not lost but pyknotic and there is diminished Nissl bodies in these neurons as well as the remaining CA1 neurons.*

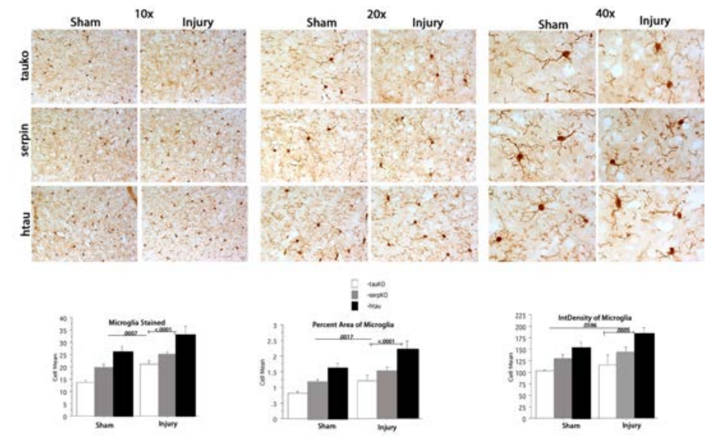

Figure 4: effect of Serpin KO or huTau alone on microglial reactivity*. Different magnification of microglia in the SL of the hippocampus are shown. Analysis of microglia show that rmTBI has robust long lasting effects on chronic microglial reactivity, but no effect in serpin KO. Since we don't have data on the cross yet, we don't know if there is exacerbation of hutau effects by serpinKO, but these data show that serpinKO alone without tau overexpression has no significant long lasting effects on microglial activation.*

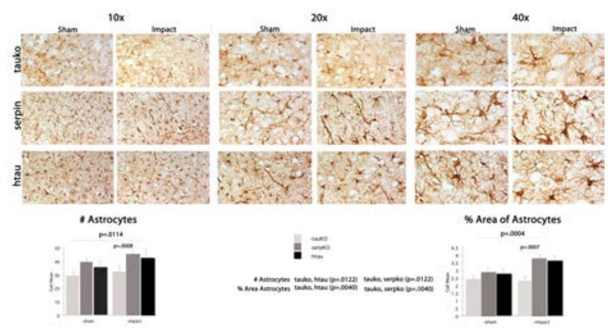

Figure 5: Effects of SerpinKO vs human tau on astrocytes after rmTBI*. Interestingly, despite no differences in sham treated animals between the three strains, relative to wildtype there was a robust increase in GFAP with both Serpin KO and rmTBI. This is very exciting data, demonstrating a direct role of C1q in dysregulation of GFAP after injury that can be independent of overexpression of human tau.*

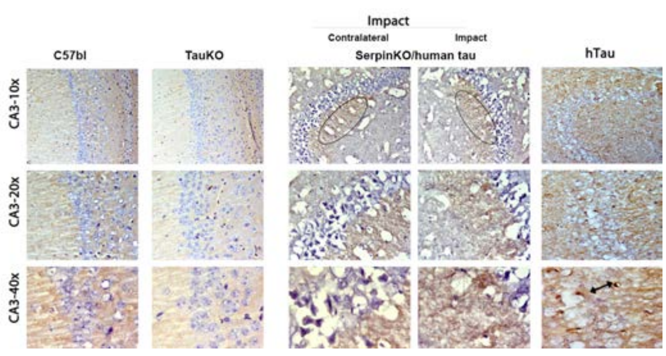

Figure 6 TBI alters neuronal distribution of the tau kinase c-jun N terminal kinase JNK after TBI in human tau mice with SerpinKO. *The normal distribution of phosphoJNK is shown in C57bl and TauKO mice (left columns). The right column shows activation of JNK in hutau mice (without injury), illustrating increased JNK in the CA3 neurons and in dendrites. In response to injury and in the presence of SerpinKO. There is increased bilateral staining in the dendrites in the stratum lacunosum after injury (shown in oval in upper panels). The higher magnification photos shown in lower panels illustrate increased pyknosis on the contralateral side and increased dendritic staining on the injured side. We will further explore whether the increase staining in the SL is injury dependent as we obtain more brains and also examine with confocal microscopy to better examine MAP2(dendritic) colocalization.*

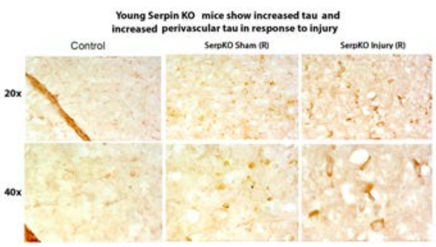

Figure 7 ***Tau after TBI in Serpin KO (without human tau).*** *Injury increases mouse tau staining in Serpin KO alone. We do not yet have data on tau staining after TBI in hu tau mice as the tissue was insufficiently fixed. However, injury in serpinKO alone increase perivascular tau (mouse tau) in the absence of the human tau transgene. These data are very exciting and we are looking forward to determining the interaction between serpin KO and hu tau on distribution of tau after TBI.*

We also assessed the plasma after mild repeat TBI in human tau tg mice to assess TBI glial biomarkers in brain derived plasma EVs (Figure 8-9). Figure 8 shows that repeat mild TBI reduced C1q, but acute TBI in humans increased plasma EV C1q. This suggests that there may be selective disruption of trafficking of C1q containing exosomes out of the brain. We also examined astroglial markers. We observed increased aquaporin and GFAP in brain derived plasma exosomes after injury (Figure 9).

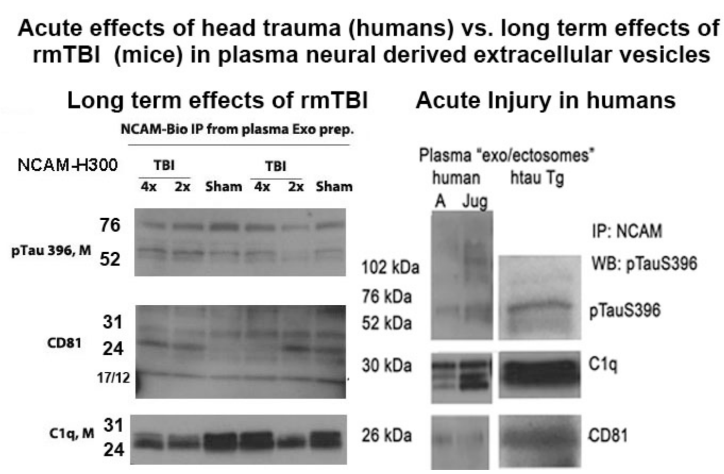

Figure 8: C1q and tau in Brain derived plasma exosomes after acute head injury in humans and long term effects after repeat mild TBI in mice. We subjected htau tg mice to repeat mild TBI (rmTBI) and, after collecting plasma, purified the neural exosomes with exoquick & NCAM IP, confirming extraction using CD81, Alix.. We could detect increased human tau in the neural exosomes in the plasma samples, in both acute severe head injury on humans and even three months after repeat mild head trauma in hutau tg mice showing that exosomal tau is released after head injury independent of severity. We also assessed C1q which is observed in exosomes. Thre months after repeat mild head traums, injury was associated with reduced plasma neural EV C1q, which may indicate that injury induced C1q may be retained in the brain due to reduced exosomal release from the brain. In contrast severe acute trauma shows increases in exosomal C1q.

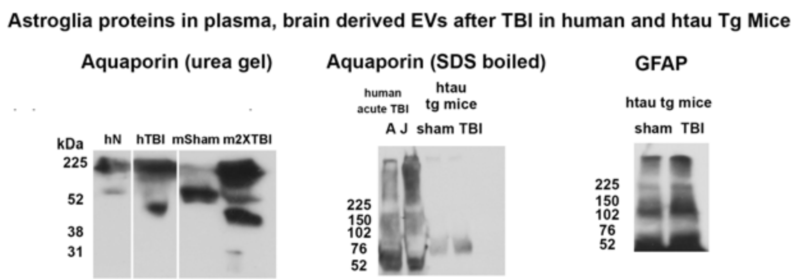

Fig 9. Long term effects of mild repeat brain trauma vs acute head trauma in humans on glial proteins in Plasma brain derived EV. *Total plasma EV from the human (hTBI) and htau mice was after sham (mSham) or 2x repetitive mild TBI and analyzed by Western on urea gel-left or after boiling with SDS and lower protein levels. The monomer is 31 kDa, but the protein has a great tendency to aggregate. Using a urea gel did not reduce aggregation, but lower less protein did. Nevertheless robust effects of injury were seen on Aquaporin. The left blot shows increases in large aggregates (225 kDa) after brain trauma in mice or humans compared to Normal human (hN) and sham mouse htau groups. The middle blot using SDS and lower protein also showed increased aquaporin after head trauma in both human and mice but the aggregates were absent in the mice. The right graph sows increase in GFAP in neural derived plasma exosomes after head trauma, which was also seen in all aggregate forms as well as the monomeric form (52 kDa)*.

What opportunities for training and professional development has the project provided?

- We have trained new undergraduate students (Ms. Alexandra Shambayate, Danielle Tran, Casey Collet and Frances Relampagos) under a research course (199).
- We have trained Eun Young Ko, a post baccalaureate attending medical school next FALL, who has obtained exceptional expertise working with these models.
- Our new postdoctoral fellow is now trained to conduct all the (in vivo) procedures in this proposal and serve a more supervisory role on the project. He has analyzed all the behavior to date, assisting in training work study and 199 students, and assisted in randomizing groups.

- **How were the results disseminated to communities of interest?** *NOTHING TO REPORT*
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *During the next reporting period, in order to accomplish the goals and objectives, we plan to breed more animals at once to keep on track for the three year period. That is although subtasks in production and testing of the mice will be delayed, we should be able to catch up by the third year with less staggering of mouse groups (producing more litters at a time). We will continue to trained undergraduate students.*

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - The impact of the findings so far is that young mice with the human gene called ‘tau’ that causes the pathology associated with brain injury show subtle differences in acute responses to TBI, despite have no neurology or behavioral problems and this is exacerbated if they have a gene that stimulations the inflammatory pathway called “Complement Cascade”. This

demonstrates that high levels of tau are sufficient to cause mild problems in response to brain injury and that this is worsened by chronic inflammation. This subtle acute response is transient and the animals fully recover and adapt, but with time the eventual failure to adapt to chronic inflammation may lead to more severe problems.

- **What was the impact on other disciplines?**
  - *This study may have an impact on understanding mechanisms of inflammation in other tauopathies (FTD, or Alzheimer's), particularly in overlap on biomarkers and role of glia. I suspect that it will overlap with mixed dementia, which may also have similar perivascular tau, which has not been looked at.*

  - **What was the impact on technology transfer?**

  - *This study may identify new biomarkers (AQP4 and GFAP) for TBI, which UCLA is applying for a patent for with Greg Cole and myself as coinventors.*
-
- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**. *NOTHING TO REPORT*
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - *There is 100% lethality when mouse tau is present in the serpinKO x tau crosses. We resolved this after dozens of litters by removing a copy of mouse tau. This will not resolve the delays in completing the studies for the cross, which can only be resolved by adding another year of funding or no cost carry over (if I can get a benefactor to donate). Similarly, we have had delays in getting enough of the C5a tau bigenics because of insufficient breeding pairs. However, there are no issues with viability, and we have resolved this issue by putting more breeding pairs together and a ratio of 3 Females to 1 male in the cage.*
   - **Changes that had a significant impact on expenditures.** We have large animal costs related to the rederiving the animals and problems with breeding and the large amount of staff needed to finish the TBI and behavioral studies and analyze the tissue as the animals needed are finally aged out. We will want to apply for a one year continuation so that we can finish aging all the animals out and analyze everything. It would be a waste of resources to kill the animals before they are aged out to complete the study. The new post doc who we were finally able to recruit this year, is of immense help and it will be important to keep him to complete the project.
   - **Significant changes in biohazards or select agents. N/A/**
   - **Significant changes in use or care of human subjects. N/A**
   - **Significant changes in use or care of vertebrate animals. NO**
   - **Significant changes in use of biohazards and/or select agents. N/A**
6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
   - **Publications, conference papers, and presentations** *NONE*
   - **Journal publications.** *NONE*
   - **Books or other non-periodical, one-time publications.** *NONE*
   - **Other publications, conference papers, and presentations.** *NONE*
   - **Website(s) or other Internet site(s)** *NONE*

- **Technologies or techniques**
  We are developing techniques to assess Plasma Extracellular Vesicles derived from the brain that may monitor inflammation related to TBI. Currently we are using human samples from another grant, and we can apply this new technology to the mouse models in this study.
- **Inventions, patent applications, and/or licenses** *NONE*
- **Other Products**v*NONE*

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

Name:	*Peter Kim*
Project Role:	*Senior Research Associate 2*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*12*
Contribution to Project:	*Mr. Kim manages the colony and breeding and works with the PI to conduct the TBI. He genotypes mice and ensures that the appropriate number are bred for the DOD project and communicates weekly about progress. He is also responsible for overseeing the work of undergraduate students.*
Funding Support:	*N/A*

Name:	*Paul Denver*
Project Role:	*Post Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*1.75*
Contribution to Project:	*Dr. Denver is involved in participating in all aspects of analysis and working with the PI to supervise the completion of the studies and writing of the papers..*
Funding Support:	*N/A*

Name:	*Anna To*
Project Role:	*Senior Research Associate 1*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*4.9*
Contribution to Project:	*Ms. To assists the PI and Mr. Kim in euthanasia, dissections and behavior. She has now left to attend Pharmacy school at UCSD*
Funding Support:	*N/A*

Name:	*Eun Young Ko*
Project Role:	*SRA1*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*0.15*
Contribution to Project:	*Ms. Ko is a pre medical student conducting experimenta and analyze behavior*
Funding Support:	*N/A*

Name:	*Jessica Obajtek*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*1.64 months (no cost)*
Contribution to Project:	*Ms.Obajtek working on the project caring for the mice.*
Funding Support:	*N/A*

Name:	*Edmond Teng*
Project Role:	*Associate Clinical Professor*
Researcher Identifier (e.g. ORCID ID):	*eteng2*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*TBI methodology/He has now left to work at Genentech in charge of Alzheimer's clinical trials.*
Funding Support:	*None*

Name:	*Andrea Tenner*
Project Role:	*Director of MIND institute UC Irvine*
Researcher Identifier (e.g. ORCID ID):	*andreatenner*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*Provided C5a Tg mice and assisting in recovering embryos and troubleshooting rederivation of the colony at UCLA*
Funding Support:	*T32 AG000096 "Training in the Neurobiology of Aging"* NIH NIA (PI, C.W. Cotman, Project Leader - A.J. Tenner) 5-01-14 through 4-30-19 $250,000 P01 AG 00538 "Behavioral and Neural Plasticity in the Aged", Project Neuroprotection and neuroinflammation induced by complement proteins and receptors $800,000 5-01-14 through 4-30-19

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - *No*
- **What other organizations were involved as partners?**
  - *N/A*
- **Personnel exchanges**
  - *N/A*

8. **SPECIAL REPORTING REQUIREMENTS**
    - **COLLABORATIVE AWARDS:** *N/A*
    - **QUAD CHARTS:** *N/A*

9. **APPENDICES:** *NO APPENDICES.*